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Isolation, taxonomy and ecophysiological characterization of endophytic fungi from *Ambrosia artemisiifolia*

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Ambrosia artemisiifolia (common ragweed) causes a serious health and environmental problems all over Hungary. Therefore the knowledge of its ecology, life-style and spreading is essential. Common ragweed can live with endophytic fungi in mutualistic relationship, without they causes negative symptoms to ragweed, although these fungi belong to plant pathogen genera such as *Alternaria*, *Fusarium*, *Mucor* and *Penicillium*. The advantage of them is that can help the host plant to uptake nutrients such as phosphorus and nitrogen, and secrete toxic alkaloids to protect the host plants against herbivores.

The goals of this research were to isolate and identify endophytic fungi from roots, stems and leaves of common ragweed deriving from typical sandy soil habitat types of Southern Great Hungarian Plain: grassland, field and abandoned field. Furthermore we described the most common endophytic fungal genera, which could help the life of the *Ambrosia* plants, from different habitats (field, abandoned field, lawn and roadsides). In addition we characterized the fungal isolates, measured their secreted enzymes: cellobiohydrolase, xylanase, β-1,3-glucanase, laccase, β-glucosidase, trypsin, chymotrypsin, cellulase, exochitinase, lipase and pectinase.

Endophytic fungi were isolated from symptomless *Ambrosia* roots, flowers and seeds from different habitats in Szolnok and near Maros in 2010, Kiskundorozsma and Ásotthalom in 2011. We made culturing from roots, leaves and flowers of common ragweed after surface sterilization and described the fungal colonies (105 colonies) at least in genus level. Most frequently *Penicillium*, *Mucor* and *Fusarium* species were isolated.

For the molecular identification, total DNA was extracted from the growing cultures belonging to distinct filamentous fungal genera. The fungal strains were identified by morphological characters and by ITS sequence analysis.

We sequenced the ITS region of 23 fungal strains from different genera and the following species were identified: *Absidia repens, Alternaria alternata, Fusarium chlamydosporum, Fusarium oxysporum, Fusarium redolens, Fusarium solani, Fusarium subglutinans, Meyerozyma guilliermondii, Mucor circinelloides, Penicillium aurantiogriseum, Pithomyces chartarum* - teleomorph: *Leptosphaerulina chartarum.*

In summary we can say that *Alternaria* and *Fusarium* species were the most abundant in flowers and seeds also, not only in the roots. *Alternaria* spores, linked to any part of the plant: adhered and germinated, could be seen by microscopical investigations. We identified *Alternaria* from *Ambrosia* seeds after aggressive surface sterilization, at first time. The activity of extracellular enzymes (laccase, cellobiohydrolase, exochitinase, β-1,3-glucanase, β-glucosidase, trypsin and lipase) activity of the fungal strains (*Alternaria*, *Fusarium*, *Penicillium* and *Mucor* genus) were also examined copper sulphate containing glucose minimal liquid medium. We experienced more increasing activity of enzyme activities in the case of the isolates belonging to the *Alternaria* and *Penicillium* genera. We ascertained that the endophytic fungal isolates could secrete cellobiohydrolase, xylanase, β-1,3-glucanase, laccase, β-glucosidase, trypsin, chymotrypsin, cellulase, exochitinase, lipase and pectinase enzymes.

Our data can contribute to more knowledge of endophytic fungi, and can help to the development of biocontrol techniques, which can solve the environmental friendly reduction of *Ambrosia artemisiifolia*.

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The molecular mechanism of sulfanilic acid biodegradation

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Sulfanilic acid (SA) is produced by the industry in large amount and – as a consequence of careless handling - it often appears in the wastes and environment. Because of its toxic properties, it should be eliminated from the natural milieu. Biodegradation is a natural environmentally sound solution for detoxification of our ecosystems.

Novosphingobium subarcticum SA1, a strain isolated from a contaminated industrial area, is a strictly aerobic chemoheterotrophic bacterium with astonishing metabolic versatility. Out of numerous aromatic compounds it can utilize sulfanilic acid as a sole carbon, nitrogen and sulfur source. Our previous studies identified the enzymes involved in the degradation of sulfanilic acid. The enzymes taking part in the

first hydroxylation reaction might be part of a membrane associated protein complex, which would be a notable advantage if we consider that they are participating in the bioconversion of a highly toxic substrate.

Recently, we sequenced the genome of this strain by new generation genome sequencers. Analysis of the genome sequence revealed that the sulfanilic acid catabolic genes are found in separate operons. Further studies on the promoters of these operons disclosed a well -regulated system which is sensitive to the nutrition, growth conditions.

Whole cell transcriptional analyses have been recently performed, which confirmed the previous findings. Additionally, several new SA inducible genes were also identified, which are likely related to the sulfanilic acid metabolism: their gene products might participate in the uptake of sulfanilic acid, in the increased export of the sulfate released from sulfanilic acid. Moreover, elevated expression of iron transport proteins were also observed, which might be a concomitant of highly expressed iron containing enzymes.

Our results suggest a well-regulated complex pathway for degradation of sulfanilic acid, in which the essential step is made by a sensitive membrane associated complex responsible for the coupled uptake and conversion of the substrate.

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Selenium- and zinc-induced stress responses in *Arabidopsis thaliana* and *Pisum sativum*. Possibilities of biofortification

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Cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), mercury (Hg) and arsenic (As) are worldwide the main contaminant metals. Their long-term deposition in soil can lead to accumulation, transport and biotoxicity because of their mobility and bioavailability. Some plants are able to take up heavy metals from soils offering possibilities for phytoremediation. However, most of the plants are sensitive to the excess of heavy metals. Zinc is an essential element for plants, animals and humans and plays necessary role in e.g. the enzyme activation, protein synthesis and carbohydrate, nucleic acid and lipid metabolism. Compared to zinc as a heavy metal, selenium (Se) is a metalloid element with large similarities to sulphur. Selenite and selenate are the main inorganic Se species present in soil and are easily processed or transferred. Selenium's necessity for plants is still questionable, on the other hand, for humans and animals it is a crucial trace element.

The main goal of this study was to investigate the short- and long-term effects of Zn and Se treatments on *Arabidopsis* morphology and the involvement of the hormonal and signalling system in this process. Modern agriculture must pay attention to the correct essential nutrient intake, since plant-based foods should be sources of all natural minerals, which are necessary for animals and humans. Therefore, also biofortification studies were carried out using pea plants.

In *Arabidopsis*, higher Se concentrations reduced primary root development, which can be an adaptation process of the plant. This reorientation of means from development for protection mechanisms ensures a better survival. Exposure of selenium disturbs protein synthesis, directly leading to cell death in the primary root meristem and growth inhibition. The hormonal balance of the root system is also affected by selenium. The Se-induced hydrogen peroxid (H_2O_2) can reduce auxin-responsive gene expression during early development, while nitric oxide (NO) inhibits auxin transport in older roots and the decrease of root auxin level results in growth inhibition. Cytokinin responsive gene expression was also enhanced by Se, which lead to growth inhibition. The selenite-induced enhancement of ethylene biosynthesis may cause cell death resulting growth hindrance. There is no regulatory link between ethylene and NO under Se exposure. The optimal level of H_2O_2 is necessary for Se tolerance and NO overproduction in *Arabidopsis* roots ensures Se tolerance.

In pea plants, results showed that selenium and zinc caused very different alterations in the development and morphology of pea plants: since Se inhibited the growth, brought forward and shortened the flowering period, Zn treatment proved to be advantageous with the increase of morphological parameters, resulted in the same long flowering period and crop production as it was observed by the control. The enzymatic background reflects to different adaptation strategies as response for the added treatments. Both selenium and zinc were effective in the stimulation of pesticide-dependent glutathione S-transferases and altogether, the shoot system was more sensitive, the activity of the antioxidant defense system was elevated. ICP results present an improved uptake of selenium and zinc in the new seeds, however the accumulation of selenium was more pronounced. Both elements accumulated in the root system at the highest level, furthermore in the case of Se this accumulation was ten times higher compared to the shoot data.

Based on the results, we could point out the effect of Se/Zn excess on the development of *Arabidopsis* and pea plants. The results can contribute to the better understanding of the hormonal and signalling background mechanisms of stress-induced morphogenic responses. Our biofortification studies on the food plant, pea may offer a solution against nutrient deficiences, since the accumulation of the added treatments did happen and were presented in the crop.

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